SARCOMA PRODUCED BY SUBCUTANEOUS INJECTIONS OF OVERHEATED COTTON-SEED OIL INTO MICE.

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BURROWS, Hieger and Kennaway (1932) announced that fats commonly used as vehicles for subcutaneous injection were under investigation as far as the production of tumours of the connective tissue is concerned. Three tumours occurred in a group of ten control mice injected with unheated lard in Burrows' (1932) experiments, but the author was suspicious that some confusion of animals or substances injected had occurred. Tumours did not arise in any other group of the injected mice, and the positive result could not be reproduced after complete precautions had been taken.

Peacock (1933) injected fowls with a solution of dibenzanthracene in lard into the right breast, and with lard only into the left breast. Tumours were observed at both sites.

Andervont (1934) found no new growth in 190 control mice injected with lard alone after 189 days.

Barry and Cook (1934) described tumour-like tissue in 2 of 10 rats following subcutaneous injection of lard after 385 and 462 days. The mass consisted of necrotic matter, granulation tissue, and some spindle-celled areas which were not considered to be malignant.

The experiments of Burrows, Hieger and Kennaway (1936) yielded not a single tumour in 216 mice after the injection of fats, fatty acids, unsaponifiable materials and liquid paraffin. In rats, however, the injection of lard alone resulted in 5 spindle-celled tumours out of 107 rats. Three more tumours were observed, one after the injection of lard with oleic acid, another after the injection of lard with theelin, and another after the injection of olive oil with arsenious acid. More than a year elapsed before the tumours appeared. Injections of lard, heated at 340–360° C. for two hours in a stream of nitrogen gave rise to two spindle-celled tumours in rats after 509 and 608 days. No tumours were obtained in mice. Three peritoneal tumours have been observed after the intraperitoneal injection of olive oil, but the authors consider it unsafe to assume that the tumours were the result of the injection.

Roffo (1938, 1939) described papillomata, adenocarcinomata and sarcomata

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of the stomach, and mesenteric sarcomata, obtained by the feeding of vegetable and animal fats heated at 350° C. for half an hour.

Domagk (1939) attempted to elicit gastro-intestinal tumours in mice by adding various apparently unheated fats to the diet. Only a few mice responded with tumours or glandular hyperplasia. These lesions were not found in the controls.

Beck and Peacock (1941) found that fats, heated under conditions that might occur in domestic cooking (repeatedly heated at 200–220° C. for a total of 12 hours), did not produce carcinoma or sarcoma of the stomach, but interfered with the metabolism of vitamin A and produced papillomata, bordering on malignancy, as a result of vitamin A deficiency, in rats fed on a diet which was adequate for controls, but not rich in vitamin A. The effect of overheated fats, as in the experiments of Roffo, is now under investigation.

Mice have been chosen for the injection of overheated cotton-seed oil, because they do not respond with the production of sarcoma as easily as rats do.

EXPERIMENTS.

Three groups, each containing 12 mice, were subcutaneously injected with 0·5 c.c. cotton-seed oil into the right flank.

In Group 1 the cotton-seed oil was heated at 340–360° C. for an hour, in the presence of air in the fume cupboard.

In Group 2 the cotton-seed oil was repeatedly heated at 200–220° C. for a total of 12 hours.

In Group 3 unheated cotton-seed oil was injected.

A new syringe and needle were used for the injections, to be sure that the cotton-seed oil was not contaminated with carcinogenic substances.

RESULTS.

Only those mice which survived the latent period of the earliest tumour (414 days) have been taken into account.

In Group 1 six mice survived. In every mouse examined post-mortem, a cyst was found containing a brown liquid similar to the overheated cotton-seed oil injected. The cyst was palpable in vivo. The first tumour, (Mouse 1866) appeared at the site of injection 414 days after the start of the experiment, and seemed to arise at the cyst wall. The mouse was killed 43 days later, by which time the tumour had penetrated into the peritoneum. Histological examination by my colleague, Dr. L. Woodhouse Price, revealed a spindle-celled sarcoma with an intercellular reticulum. The second tumour appeared at the site of injection 538 days after the start of the experiment (Mouse 1869). The tumour was very close to a similar cyst containing brown fluid, and proved, histologically, to be a spindle-celled sarcoma with a fine reticulum. No secondaries were observed in either mouse, and grafts have not been undertaken.

In Group 2 three mice only survived. A cyst was usually found containing the injected cotton-seed oil, except in the mice which died after twelve months. No tumours occurred. The small number of surviving mice makes it necessary to repeat the experiment.

In Group 3 ten mice survived, and six lived for more than 560 days. Neither cysts nor tumours were observed in this group.

DISCUSSION.

Before injection a sample of the overheated cotton-seed oil (340–360° C.) and of the repeatedly heated cotton-seed oil (200–220° C.) was spectroscopically examined. The spectrum showed only a general fluorescence and no bands, proving that none of the known carcinogens was present, and proving also that no contamination with carcinogens could have occurred. The heated cotton-seed oils, after having been purified chromatographically, contained a substance which gave a red colour when added to antimony trichloride. The colour was different from the red of the cholestadiene reaction.

Besides the production of sarcomata by overheated cotton-seed oil, three facts are of interest :

First, the great persistence of the depot of overheated cotton-seed oil. Second, the nearness of the tumour to the depot of overheated cotton-seed oil. Third, the long latent period.

These three facts can be correlated to each other.

Peacock (1933) described the topical conditions of tumour and tar depot in fowls. The tumour never arose in immediate contact with the main tar depot, but at some distance from the smaller depots in the direction of the lymph stream.

Peacock and Beck (1938) observed the same fact for benzpyrene tumours, and suggested that the tumour occurred at a point at some distance from the depot where the optimum rate of absorption was maintained for an optimum time. It seems obvious that the sarcoma-producing substance acts while it is being absorbed. In the case of overheated cotton-seed oil, the tumour starts very near the depot ; this suggests that, either the rate of absorption is very slow, coinciding with the persistence of the depot, or that the concentration of the tumour-producing substance in overheated cotton-seed oil is very low.

The long latent period can also be explained by the foregoing facts. Assuming that the tumour-producing substance acts on its way to absorption, the process of inducing sarcomata appears similar to carcinogenesis as a result of painting. In the latter case, the carcinogen is applied every few days. In the case of sarcomagenesis, we have a more continuous stream of tumour-producing substances being absorbed through the tissues. We know that a dilution of the carcinogen in painting experiments results in a prolongation of the latent period. It seems justifiable to conclude, as far as sarcomata are concerned, that a slow rate of absorption or a low concentration of the tumour-producing substance brings about the conditions for a long latent period.

SUMMARY.

(1) Subcutaneous injection of overheated cotton-seed oil (340–360° C.) produced sarcomata in two of twelve mice, at the site of injection, after 414 and 538 days.

(2) Repeatedly heated cotton-seed oil (200–220° C.) and unheated cotton-seed oil did not produce tumours.

(3) Secondary findings : (*a*) A long persistent depot of overheated cotton-seed oil, (*b*) nearness of the tumour to this depot, and (*c*) a very long latent period, are correlated to each other.

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THE OCCURRENCE OF INFLUENZA B IN SOUTHERN ENGLAND.

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SINCE the isolation of a virus by Smith, Andrewes and Laidlaw from patients suffering from influenza (1933), it has become increasingly obvious that only a part of the story was then revealed. For example, in an investigation of the 1939 epidemic in England (Stuart-Harris *et al.*, 1940) less than 30 per cent. of sera tested from patients with clinical influenza gave evidence of infection with the virus in question (now known as influenza A virus (Horsfall *et al.*, 1940). Recently Francis (1940) has described the isolation from an influenza outbreak in the U.S.A. of another type of virus which has been called influenza B virus. This virus, while producing the same range of symptoms as influenza A virus, is unrelated to it antigenically. Francis found that influenza due to virus B had been widespread in Eastern and South-eastern U.S.A. in January–February, 1940 ; further, study of sera preserved since 1936 showed that it had also occurred widely in an epidemic in California during that year.